CHAPTER 2

LITERATURE REVIEWS

2.1 General information of *Dendrobium*

Dendrobium belongs to subfamily Epidendroideae, tribe Dendrobieae, subtribe Dendrobiinae (Dressler, 1993). The genus was first described in 1800 by Olof Swartz, the generic name of this genus derived from the Greek words *dendron* which meant tree and *bios* meant life (Sheehan and Sheehan, 1994). *Dendrobium* is the second largest genus in the orchid family after *Bulbophyllum*. Without a generally accepted review of the genus, the number of valid species is uncertain, but estimates ranging from 900 to 2000 species. The number of valid species also varies depending on which taxonomist is being followed. Botanists divide the genus into closely related groups using the sections. However, they frequently disagree regarding which plants belong to which section because some species fall somewhere between sections (Baker and Baker, 1996). Rudolf Schlechter divided the genus into four subgenera and 41 sections (Pridgeon, 1994).

Orchid growers frequently refer to sections or groups of closely related plants. Some common sections that may be encountered in other literature include *Calyptrochilus*, *Dendrobium*, *Dendrochoryne*, *Desmotrichum*, *Latourea*, *Formosae*, *Oxyglossum*, *Pedilonum*, *Phalaenanthe* and *Spatulata*. *Dendrobium* growers may also refer to a group of plants by the name of the most commonly cultivated species in its group such as the “anosmum” or “nobile” group, or by a flower feature such as the “antelope” group, or a growth characteristic such as evergreen, deciduous, soft cane, or hard cane (Baker and Baker, 1996).

The genus has a wide distribution extending from Korea and Japan through the Indo-Malaysian region to Australia and New Guinea and into the Pacific Islands, with a concentration of extremely fine species in New Guinea (Soon, 1980). *Dendrobium* grows primarily in tropical to subtropical climates, but some species grow in temperate mid latitudes or at high elevation in

tropical areas (Baker and Baker, 1996). In Thailand, there are about 150 species in 14 sections, 2 species of *Bolbidium*, 10 species of *Callista*, 17 species of *Formosae*, 13 species of *Pedilonum*, 36 species of *Dendrobium*, 10 species of *Breviflores*, 9 species of *Distichophyllum*, 20 species of *Stachyobium*, 11 species of *Rhopalanthe*, 16 species of *Aporum*, 3 species of *Strongyle*, 4 species of *Grastidium* and 2 species of *Conostalix* (Seidenfaden, 1985).

Den. anosmum **Lindl.** is a widespread species from the Philippines to New Guinea, including Borneo and many island in Indonesia. It was also found in Malaya, Peninsular Thailand, Laos, Vietnam and Sri Lanka, from the sea level to 1300 ms (Seidenfaden, 1985 and Baker and Baker, 1996) and was also found in Northeastern Thailand, its Thai name is Ueang Sai Luang (Vaddhanaphuti, 2005). The plant has cylindrical pseudobulbs, 100 to 300 cms long which may be arching or pendulous, consists of nodes spacing a few inches apart. The mature pseudobulbs turn to be yellow, then silver gray with age. Their leaves are large oblong-lanceolate, 12 to 18 cms long, pointed at apex, fleshy, and glossy green. The leaves normally last a single season and fall just as the flower buds appear, the leaves should not be removed when they start to dry, as it is very easy to damage the buds forming at the leaf axils. Inflorescences arise from nearly every node on mature, leafless pseudobulbs (Baker and Baker, 1996). Each inflorescence bears 1 to 2 flowers (Baker and Baker, 1996 and Vaddhanaphuti, 2005). The flowers are normally 7 to 10 cms across, dark rose-red to deep lavender including intermediate shades. The translucent segments are darkest at base with paler tips. Color of the broad petals and the narrow sepals are the same accented with darker veins. The lip is longer than the dorsal sepal and commonly heart-shaped, hairy on the inside and usually marked with deep purple stripes in the throat. The flowers have a strong pleasant scent which reminds some of raspberries, and last about 3 weeks (Baker and Baker, 1996). Blooming season is from January to April (Vaddhanaphuti, 2005). Chromosome number of this specie is $2n$ = 38 (Lim, 1985). Cultivation conditions, growing temperature should be maintained all the year, day temperature average at 26 to 30^0C and night temperature average at 16 to 20^0C with diurnal temperature range of 7 to 11^0C, light intensity between 400 to 600 $\mu mol.m^{-2}s^{-1}$ in summer, the species prefer morning light and should be shaded at midday, humidity of 80 to 90 % in summer, and spring, dropping to near 70 % in winter. Rainfall is low for 3 to 4 months in winter. In cultivation, water should be reduced and plants are allowed to dry out between watering. They should not remain dry for extended periods. Fertilizer

should also be reduced or eliminated anytime the water is reduced. In the habitat, light is strongest in winter. Grower indicates that water should be gradually increased in late winter after the flower buds develop (Baker and Baker, 1996). This species has been a popular garden plant, often attached to trees or grown in hanging baskets. Its major attribute is the strong fragrance (Kamemoto *et al.,* 1999).

Den. parishii **Rchb. f**. is widespread in the eastern Himalayas from the Manipur region of northern India through the Tenasserim, Maymyo, and Chin Hills regions of Myanmar, across northern and eastern Thailand, through Laos and Vietnam, and into Yunnan and Gweizhou Provinces of southwest China (Baker and Baker, 1996). In Thailand, plants grow in dry deciduous forests (Baker and Baker, 1996 and Thaithong, 2000) at 250 to 1700 ms above sea level (Baker and Baker, 1996). Its Thai names are Ueang Nam Khrang Sai San and Ueang Sai Nam Khrang (Vaddhanaphuti, 2005). The fresh cylindrical pseudobulbs with persistent white sheaths (Seidenfaden, 1985), curved downward, and knotty at the numerous nodes, 10 to 30 cms long (Thaithong, 2000). The plants are up to 60 cms long and may be stout or elongated depending on growing conditions. Plants from drier regions with high light intensity are usually shorter with upright growth habit (Baker and Baker, 1996). However, the pseudobulbs of plants from shady, humid areas tend to be longer, pendant and may reach a length of 190 cms (Seidenfaden, 1985). Leaves are oblong-lanceolate, 5 to 15 cms long, stiff, leathery, and deciduous. Inflorescences, short, arise from nodes along more than 50 % of the length of the pseudobulbs after the leaves have fallen (Baker and Baker, 1996). Inflorescence bears 1 to 3 flowers (Vaddhanaphuti, 2005) or 4 flowers, with 4 to 6 cms across (Baker and Baker, 1996). Sepals and petals are purple (Vaddhanaphuti, 2005) or dark rose fading to white near the center, petals are finely toothed margins (Baker and Baker, 1996). Lip is shorter than dorsal sepal, edges shortly ciliate, with light purple edges, the dark purple center often split in two spots (Seidenfaden, 1985). The column is white. The long-lived blossoms have good substance and strong scent. Their fragrance has been variously described as like rhubarb or raspberries (Baker and Baker, 1996). Chromosome number of the species is $2n = 38$ (Lim, 1985). Cultivation conditions, this species can grow well in summer at day temperature average between 25 to 26 ^{0}C and night temperature average from 17 to 18^{0}C with diurnal temperature range of 7 to 8^{0}C, and light intensity from 400 to 600 $\mu mol.m^{-2}s^{-1}$. Good ventilation is important at all times, humidity of 80 to 85% from summer to autumn,

dropping to 60 to 70% for most other months. Cultivated plants should be kept moist while actively growing, but water should be gradually reduced after new growths mature in autumn (Baker and Baker, 1996). Flowering period is April and May (Kamemoto *et al.,* 1999 and Vaddhanaphuti, 2005).

Den. scabrilingue **Lindl.** is widespread in Myanmar, Laos and Thailand (Baker and Baker, 1996). This delightfully fragrant orchid grows at high elevations in northern, northeastern and eastern Thailand (Kamemoto *et al.,* 1999). Its Thai names are Ueang Sae, Ueang Sae Hom and Ueang Sae Luang (Thaithong, 2000). The plants are usually found in the highlands at 610 to 1220 ms above sea level. In Chiang Mai, Thailand, it can be found at 760 ms above the sea level (Baker and Baker, 1996). The plants have 10 to 20 cms long pseudobulbs (Thaithong, 2000), erect and swollen above the narrow base, they are somewhat lumpy and knobby looking. The leaves are leathery and persistent, oblong, 6 to 10 cms long, 2 to 6 leaves on distal part of pseudobulbs. The leaf sheaths are covered with blackish hairs. Inflorescences are very short with numerous flowers producing from apical and upper nodes of leafy pseudobulbs and on lower nodes of leafless pseudobulbs. There are 1 to 3 waxy flowers per inflorescence (Baker and Baker, 1996). The flowers are about 2 cms (Thaithong, 2000) or 3 cms across, sepals and petals are green to greenish white when they first open but change to white or ivory within a few days. The 3-lobe lip is yellow-green with green veins when first opens. The smooth keel starts at base of lip and separates into 3 lines at center of the mid lobe, the tips are warty and uneven, eachside of the keel split into 2 less prominent lines. The side lobes are green with red markings. The mid lobe has a yellow disk with deep orange grooves and crimson stripes. Individual blossoms last about 5 weeks, but the plant is often in bloom for many months as buds continue to form. The flowers have a sweet fragrance reminiscent of wallflowers (Baker and Baker, 1996), because of their distinct and pleasing fragrance make them a favorite flower in Thailand (Kamemoto *et al.,* 1999). Chromosome number of the species is $2n = 38$ (Tanaka and Kamemoto, 1984). Cultivation conditions, it can grow well in summer at average day temperature between 28 to 30^0C and average night temperature at 21^0C with diurnal temperature range of 7 to 9^0C. Humidity is near 80 % most of the year and dropping to near 60 % in late winter. Cultivated plants should be kept moist while actively growing, but water should be gradually reduced after new growths mature in autumn (Baker and Baker, 1996).

***Den. peguanum* Lindl.**, the compacted plants are widespread from southeastern India to Myanmar (Baker and Baker, 1996) and northern Thailand (Vaddhanaphuti, 2005 and Thaithong, 2000) at 300 to 400 ms above sea level (Baker and Baker, 1996). Its Thai name is Ueang Nang Lom (Vaddhanaphuti, 2005 and Thaithong, 2000). The plants are stout, tufted, ovoid to oblong-conical pseudobulbs, 3.6 to 6 cms long. There are 2 to 4 leaves, broadly elliptic to linear-oblong, 3.5 to 7.5 cms long, at the apex of pseudobulbs. One or more inflorescences, 2.5 to 7.0 cms long, emerge near the apex of leafless pseudobulbs shortly after leaf falling. The fragrant blossoms are clustered near the apex of inflorescence (Baker and Baker, 1996), 5 to 8 flowers per inflorescence (Vaddhanaphuti, 2005), the flowers are 1.2 cms across (Baker and Baker, 1996), sweet fragrance like honey (Thaithong, 2000). The white sepals and petals are some times flushed with purple. The blossoms have an erect dorsal sepal and sickle-shaped lateral sepals, with lanceolate, somewhat sickle-shape petals, the 3-lobe lip is tan or light brown with darker brown or purple lines. The deeply ruffled and pointed mid lobe is suffused with amethyst or purple. It has a large, fleshy, rod-like callus between the long side lobes (Baker and Baker, 1996). Chromosome number of this species is $2n = 38$ (Tanaka and Kamemoto, 1984). Cultivation conditions, this species can grow well in summer at day temperature average 30^0C and night temperature average 23 to 24^0C with diurnal temperature range of 7^0C. Humidity is near 85% in summer, dropping to 55 to 60 % in late winter. Cultivated plants should be kept moist for 5 months in summer while actively growing but should be kept dry for 5 months in winter, allowed media to dry out between waterings and especially on warm, sunny days water should be applied misting in early morning to prevent the plants from becoming too dry (Baker and Baker, 1996).

2.2 Orchid pollinia storage

Since each species of orchid blooms at different time of year, in order to make hybridization possible, keeping orchid pollinia in the refrigerator is a common practice for orchid breeders. However, proper temperature and period of storage of each species are not well documented. There are few reports on pollinia storage. Air-dry storage at 7 °C has proved successful for up to 12 months with two species and one hybrid of *Dendrobium* (Meeyot and Kamemoto, 1969). Similar success with other species of *Dendrobium*, *Vanda*, *Cymbidium* and *Arachnis* has been achieved using air-dry storage at 4 to 6 °C for the maximum of 280 days

(Shijun, 1984). Pollinia can be also kept in a small tube for 2–12 months in regular refrigerator (Songkhakul, 1983). Pollinia of *Dactylorhiza fushsii*, *Orchis morio*, *Orchis maculata* and *Anacamptis pyramidalis* could be kept at 2°C and 85 % relative humidity in refrigerator for 60 days (Prichard and Prendergast, 1989). Pollinia of *Dendrobium nobile* stored at -79 °C for 957 days survived in the presence of a chemical cryoprotectant, glycerol and ethanol mixture (Ito, 1965). However, the use of very dry storage is not advisable for all pollen. For example, Gramineae pollen is generally intolerant of desiccation. In addition, reports on the effect of short-term drying and long-term storage over desiccants in orchid pollinia were conflicting. Desiccation over silica gel reduced pollinia viability (Pritchard and Prendergast, 1989). Some orchids, *Cattleya mossiae* (Curtis and Duncan, 1947), and *Dendrobium* Lady Hamilton (Ito, 1965) appeared relatively tolerant to drying, whereas such conditions were harmful in other dendrobiums and *Oncidium stipitatum* (Meeyot and Kamemoto, 1969).

2.3 NAA on delaying fruit drop

Indole acetic acid (IAA) is probably the auxin involved in post pollination, and perhaps postharvest phenomena, because it is present in high concentrations in orchid pollinia. However, it is not clear at present whether its source is the pollinia or other parts of flower, or both. Some post pollination and postharvest phenomena were ethylene-mediated, and another unanswered question was whether production of the gas was brought about by auxin and ACC, both of which may have present and could be produced by the pollinia (Avadhani *et al*., 1994). Comparisons among several auxins showed that synthetic auxin had more pronounce effects than IAA and that α-naphtalene acetic acid (NAA) was the most effective compound (Hsiang, 1951). NAA could also induce embryo-sac formation and caused seed capsule to show sign of parthenocarpy (Zimmerman and Hitchcock, 1939). After pollinarium took place, ovaries of pollinated orchid flowers swelled and elongated (Hsiang, 1951). However, fertilization did not take place until much later. It is very common in orchid that there is a post pollination phenomenon. In *Dendrobium*, there were reports stating that fertilization took place at about 60-75 days after pollination (Sagawa, 1993). Thus, NAA has been employed to enhance fertilization in some orchid hybridization. NAA applications to the stigma and pedicel of *Cymbiudium* could increase ovary diameter, the swelling was caused by cell enlargement not number (Arditti and Flick,

1976). In *Vanilla planifolia*, applying some plant growth regurator chemicals such as IAA, NAA, 2,4-dichlorophenoxyacetic acid (2,4-D) and indolebutyric acid (IBA) could induce parthenocarpic fruit set (Gregory *et al*., 1967); and auxin-induced fruits of *Vanilla* developed faster than those initiated by pollination (Bouriguet, 1954).

2.4 Cross pollination and inheritance

Orchid is one of a few species in plant taxa that can hybridize within as well as between section, and genus. However, there is more incompatibility within the same genus. Since one genus of orchid consist of few species up to thousand species, somtime, genus is divided into sections. In *Dendrobium*, there are about 1,500 species which can be divided into 41 sections. Thus, within the same genus of *Dendrobium*, not all of the *Dendrobium* can hybridize with each other even within the same section. There was report stating that intrasectional and intersectional crosses might not always indicate the degree of relationship of species, however, a success in obtaining interspecific hybrids usually indicates a close relationship. Kamemoto *et al.* (1999) presented in the book '*Dendrobium* Breeding in Hawaii' that section Spatulata and *Phalaenanthe* are easily crossed (Figure 1).

On the basis of ease of crossability, the *Spatulata* and *Phalaenanthe* sections appear to be closely related. Intrasectional *Spatulata* pollinations produced 53.1% fruit set, and intrasectional *Phalaenanthe* resulted in 100% fruit set. Intersectional pollinations between *Spatulata* and *Phalaenanthe* resulted in 79.1% fruit set. Intersectional pollination of *Latourea* with *Spatulata* and *Phalaenanthe* produced 28.9% and 28.4% fruit set, respectively. Crosses between the *Formosae* section and both of *Spatulata* and *Phalaenanthe* yielded the successful from 13.0% and 33.3%, respectively. While the percentages of successful crosses obtained from crossing of *Dendrobium* with *Spatulata* and *Phalaenanthe* were 6.2% and 9.1%, respectively, therefore the *Latourea* section appeared to be more closely related to the *Spatulata* and *Phalaenanthe* sections than to the rest of the sections investigated (Kamemoto *et al*., 1999). In around 1970s, there was a revival of interest in intersectional breeding between the *Latourea* and *Formosae* sections and the popular *Phalaenanthe* × *Spatulata* sections with some promising hybrids being produced with *Den. macrophyllum*, section *Latourea* whereas breeders in Singapore-Malaysia and in Hawaii have concentrated on only two sections, *Phalaenanthe* and *Spatulata*, in hybridization, while in

temperate countries horticultural interest is focused on the member of *Callista* and *Dendrobium* (Soon, 1980).

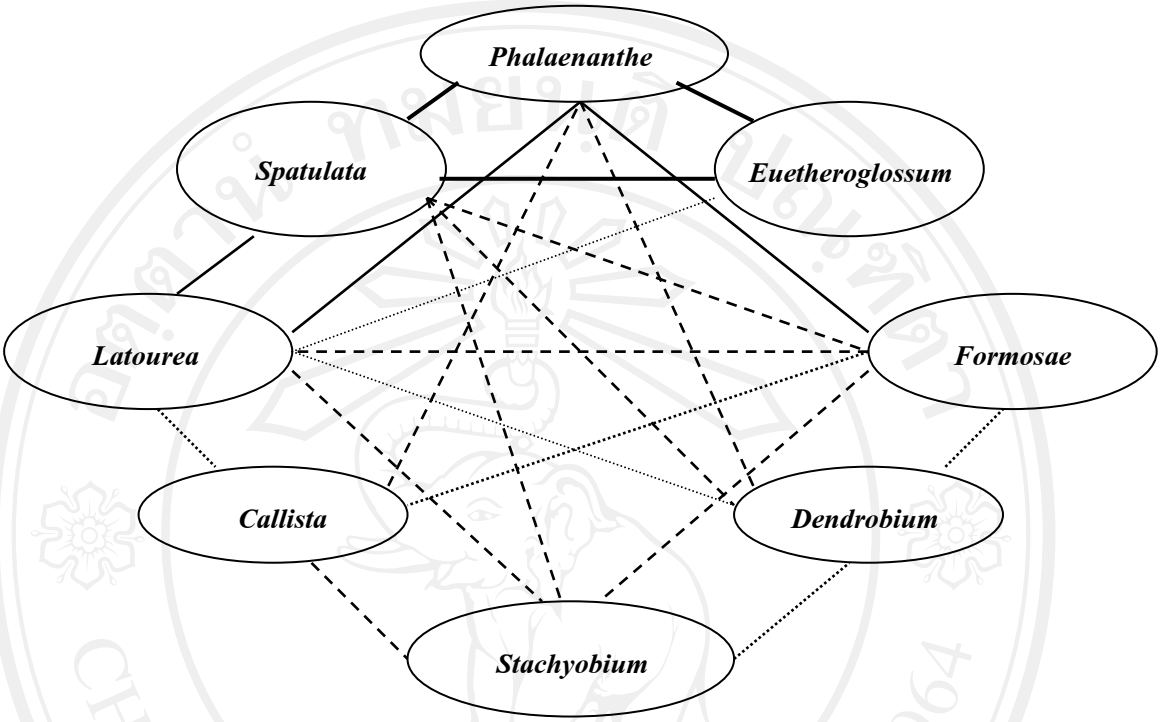

Figure 1 Crossability of species between sections in the genus *Dendrobium*.

Reproduced from Kamemoto *et al*. (1999)

In the past, professional plant breeders could work independently of the geneticist; it was not necessary for them to understand the principles of genetics. University and government plant breeders were the first who applied the understanding of inheritance for plant improvement. Conventional breeding relies upon selecting plants with different gross visible characteristics. Plant improvement has become more difficult today, because most of easy breeding has already been accomplished. An increasingly sophisticated approach would be needed in order to make progress in more difficult areas or add on special character (Griesbach and Kooperwitz, 2005). Qualitative characters inheritance such as flower color, has been determined, usually, the final

color produced by the flower is determined by one or other of three pigment classes either alone or combination. If no pigment is present to alter the absorption of light, the color of the flower is white, whether the light is reflected or transmitted (Vogelpoel, 2005). For example, white-flowered *Cattleya* mutants were separated into two classes, the true *alba* class, with absolutely no pigmentation and the false *alba* class, more properly called *albescent*, with only few pigments present (Griesbach and Kooperwitz, 2005). It is important to understand the biochemical basis of these mutations and how they can be used to create the elusive blue color. Red through blue gives the result of flavonoid pigments. Two groups of flavonoids are the anthocyanins and the co-pigments, the color of anthocyanins depends on the pH, while co-pigments are colorless to light yellow (Griesbach, 2005). When breeding for color changes, it is important to appreciate that the genes for anthocyanin and carotenoid pigments are transported through gamete cells by different pathways. The genes responsible for anthocyanin synthesis are located in the chromosome of the cell nucleus where two copies of each gene are present in the normal diploid parent. Since the production of the male and female gamete cells involves equal division of all nuclear chromosomes during meiosis, one copy of each gene is transmitted to the haploid cell of the pollen and ovules. The mode of inheritance is therefore bi-parental. By contrast, the genes responsible for yellow carotenoid and chlorophyll pigments are located in the primitive chromosomes within the chromoplasts and chloroplasts respectively. Therefore, plastid DNA appears to be exclusively transmitted by the female or seed parent (Vogelpoel, 2005). Fragrance was one of the characteristics that breeder would like focus on (Groom, 1995). There were two types of main scent, 'spicy-floral scent', attributable primarily to eugenol and cinnamic alcohol, and 'aromatic-floral' aspects of benzyl acetate predominate in the other group (Kaiser, 1993). There are few reports on fragrant inheritance. Fragrance of *Disa* is inherited through seed parent whereas in *Paphiopedilum*, fragrance can be transmitted by pollen parent (Kalina, 1992), which is similar to *Phaius tankervilleae* (Inpar, 2003).

Additional, some quantitative characters of orchids have been reported. *Den.* Nestor, progenies from the cross between *Den. anosmum* and *Den. parishii*, were studied on their phenotypic variation, some quantitative characters such as flower width, dorsal sepal length, petal length and lip length, which found to produce their means more than those from *Den. parishii,* but were significantly less than those from *Den. anosmum*. As for qualitative characters, petal color

and lip color, all of the progenies presented the petal color at the mid-parents value, more deep color than *Den. anosmum* but less than *Den. parishii*, all of them had more deep color of lip than *Den. anosmum.* Color of the lip was only one character that affected by reciprocal crossing, progenies from the cross of *Den. parishii* as fruit parent presented more significantly deep color than those from *Den. anosmum* when used as fruit parent (Yotsoi, 2006).

In many cases an observed frequency distribution deviated obviously from normality, thus statistics that measured the nature and amount of devaition were useful. Sokal and Rohlf (1981) explained in the book 'The Principle and Practice of Statistic in Biological Research' that there were two types of deviation from normality. One was skewness, which was another name for asymmetrical means that one tail of the curve was drawn out more than the other. In such curves the mean and the median did not coincide. Curves were called skewed to right or left depending upon whether the right or left tails were drawn out. The other type was kurtosis, which a more complicated change in distribution. If a symmetrical distribution was considered having a center, two shoulders and two tails, kurtosis described the proportions found in the center and in the tails in relation to those in the shoulders. A leptokurtic curve had more items near the center and at the tails, with fewer items in the shoulders relative to a normal distribution with the same mean and variance. On the other hand, platykurtic curve had fewer items at the center and at the tails than the normal curve but had more items in the shoulders. A bimodal distribution was an extreme platykurtic distribution. The conventional sample statistic for measuring skewness, g_1, and kurtosis, g_2, to present population parameters, γ_1 and γ_2. The formulas for g_1 and g_2 involve moment statistics. A central moment in statistics, as in physic, is $(1/n)\Sigma^n(У - \bar{У})^r$, n = number of all classes, У = class value, $\bar{У}$ = average of class values and r = order number of central moment, the average of the deviations of all items from the mean, each raised to the power *r*. The first central moment, $(1/n)\Sigma(У - \bar{У})$, was always equals zero. The second moment, $(1/n)\Sigma(У - \bar{У})^2$, was the variance. The statistic g_1 was the third central moment divided by the cube of the standard deviation, $(1/ns^3)\Sigma(У - \bar{У})^3$ and g_2 was 3 less than the fourth central moment divided by the fourth power of the standard deviation, $(1/ns^4)\Sigma(У - \bar{У})^4 - 3$. Just as the sample variance had to be corrected for bias by dividing $\Sigma(У - \bar{У})^2$ by $n - 1$ rather than n, so sample g_1 and g_2 needed to be corrected to allow for similar bias. Additional, in a normal distribution both of population parameters, γ_1 and γ_2 were zero. A negative g_1 indicated skewness to the left whereas positive g_1

indicated skewness to the right. A negative g_2 indicated platykurtosis, while a positive g_2 showed leptokurtosis. Thus, a repulsed distribution would have a positive g_2, but a clumped distribution should have a negative g_2.

2.5 Identification by RAPD technique

DNA markers have been used to manipulate marker-assisted selection, and to guide the introgression of target genes from related species (Wolff *et al*., 1994). Early DNA-based techniques were mostly not applicable to conservation studies because the amount of DNA used required the destructive sampling of large amount of plant tissue. However, this situation changed dramatically with the invention of the polymerase chain reaction (PCR), which allowed the use of minute amount of DNA because it produced large numbers of copies of the fragments of DNA under study (Fay, 1999). An alternative technique for identifying molecular markers called random amplified polymorphic DNA (RAPD) has been developed. In this method, a single arbitrary primer is used and then DNA is amplified using PCR. Amplified DNA could easily be separated on an agarose gel by electrophoresis (Williams *et al*., 1990). This technique has been recently employed in molecular studies.

RAPD technique has been employed to differentiate and evaluate quite a number of orchid genera and species. There was one paper on *Dendrobium*, using RAPD to evaluate the relationship of hybrids and their parents. In intersectional hybrids, section *Phalaenanthe* x section *Formosae*, of *Dendrobium* were evaluated using 21 decamer primers and RAPD technique. It was found that 7 primers, OPF 01, OPF 02, OPF 03, OPF 04, OPF 05, OPF 06 and OPD 03, could yield good polymorphic pattern and confirm the intersectional hybrids (Inthawong *et al*., 2006)

Other than that, this technique was also used in other orchid genera and species as followed. It was used to identify thirty-six *Cymbidium* cultivars. Ten primers were tested. It was found that 78% of 132 samples presented clearly different polymorphic DNA bands and *Cymbidium* Blue Smoke could be differentiated from other cultivars when OPA 5 primer was employed (Obara-Okeyo and Kako, 1998). The interspecific and intraspecific relationships of 21 cymbidiums were analyzed by using RAPD. Twenty-two primers were used in the analysis by comparing differences of DNA patterns of all species and cultivars. The cymbidiums could be divided into two clusters based upon ecologicaltraits. One trait was temperature zone preference,

with each cymbidium preferring either an Asian or subtropical temperature zone. The group that comprised the subtropical cymbidiums was *C. aloifolium*, *C. insigne* and *C. lowianum*. Another trait was basing on physiological and morphological characteristic, which could be grouped *C. lancifolium* and *C. aspidistrifolium* by different flowering physiology and unique leaf from. The groups identified by morphological, physiological and ecological characteristic were in full agreement with those determined by RAPD analysis (Choi *et. al*., 2006).

The genetic diversity and relationships among twenty paphiopedilums and fourteen phragmipediums was determined by RAPD using 200 decamer primers, 100 primers of each UBC #2 and #7. It was found that using UBC 241 primers produced clearly distinguished *Paphiopedilum rothschildianum* from the other paphiopedilums and *Phragmipedium sargentianum, Phrag. pearcei, Phrag. Longifolium, Phrag.* Belle Hogue Point*, Phrag.* Bakara LeAn*, Phrag.* Mem. Dick Clements, *Phrag.* Don Wimber and *Phrag.* Hanne Popow (Chung *et al*., 2006).

Genetic relationship among 32 species of *Phalaenopsis* and related genera, *Doritis* and *Kingidium* was studied. Species belong to these three genera were evaluated by RAPD using six primers, OPK 10, OPD 03, OPF 01, OPF 02, OPF 09 and OPF 14. It was found that *Phalaenopsis* and related genera could be divided into 11 groups. The results of using this technique were similar to plant classification by using morphological characteristics (Taywiya *et al*., 2008). Inaddition, genetic relationship of *Phalaenopsis amboinensis*, *Phal. amabilis* and their hybribs was also identified by RAPD using OPC 07 primer. It was found that the hybrids were the result of genetic combination of their parents. The study of somaclonal variation in *Phalaenopsis* True Lady “B79-19” derived from tissue culture was studied, in 1,360 flowering somaclones, no apparent difference was found in the shape of leaves, whereas flowers in some somaclones were deformed. The RAPD data indicated that normal and variant somaclones were not genetically identical (Chen *et al*., 1998). Moreover, there was also paper that RAPD technique was used to identify the markers linked to red floral trait of *Phalaenopsis equestris*, white floral parent, red floral parent, F_1 progenies and F_2 progenies, which controlled by a single dominant gene. A total of 920 primers were used for screening markers that were related to the red floral gene. It was found that a 380 bps DNA fragment (OPQ10-380) from OPQ 10 primer linked to the red floral gene (Chen *et al*., 2001b).